# Forest Health Protection









**Numbered Report 09-03** 

March 2009

### Individual-Tree Tests of Verbenone and Green-leaf Volatiles to Protect Lodgepole, Whitebark and Ponderosa Pines, 2004-2007

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#### INTRODUCTION

Verbenone, (4,6,6-trimethyl-bicyclo [3.1.1] hept-3-en-2-one), is a known anti-aggregation pheromone of mountain pine beetle (MPB), Dendroctonus ponderosae Hopkins. "pouch" formulation has been tested to protect pine stands and individual pine trees numerous times with favorable results (Bentz and others 2005, Gibson and Kegley 2004, Kegley and Gibson 2004, Borden and others 2003, Progar 2003, Kegley and others 2003). However, this pouch did not elute sufficient pheromone over the entire flight period of MPB and had to be replaced mid-season for adequate tree protection. We tested additional verbenone formulations in protecting individual trees in 2004, 2006 and 2007. In 2006 and 2007, we also tested the efficacy of verbenone plus a green-leaf volatile (glv) pouch (50:50 blend of z-3-hexenol and 1-hexanol).

#### **METHODS**

#### **2004 Test**

We tested a new 7.5-gram verbenone pouch produced by Biota, Inc. in both lodgepole pine on Corona Ridge near Plains, Montana; and in whitebark pine near Fisher Peak in the Selkirk Mountains north of Bonners Ferry, Idaho. These areas were selected because of high levels of MPB activity (Figure 1). On Corona Ridge, we also tested the EPA-registered 5-gram pouch produced by Pherotech, Inc. Our treatments were: (1) two 5-gram Pherotech pouches per tree, changed mid-season, (2) two 7.5-gram Biota pouches per tree, and (3) control, no verbenone pouches. Fifty trees comprised each treatment. In addition, each tree was baited with standard MPB tree bait (Biota, Inc.). Verbenone pouches were stapled to the northeast and northwest sides of tree boles at a height of six to seven feet; tree baits to the north side of trees about six feet above the ground.

All treatments were installed June 22.



Figure 1. Whitebark pine mortality due to MPB on Fisher Peak, 2004.

Treatment trees were located at least 2 chains (132 feet) apart. After beetle flight, on September 21-22, trees were rated as massattack, strip-attack, pitch out, or no attack. "Mass-attack" is defined as a tree successfully attacked by MPB and killed. "Strip-attack" is a tree successfully attacked on a portion of its bole, but not killed. A "pitch out" is a tree unsuccessfully attacked; "no attack" is a tree without any attacks.

On Fisher Peak, our treatments were: (1) two 7.5-gram Biota pouches and (2) control, no verbenone pouches. Thirty-five trees were included in each treatment. MPB tree baits were placed 5-10 feet from each treated tree. Treatments were applied June 17-18 and evaluated September 23 in the same manner as the Corona Ridge test. Treatment trees were located at least 2 chains apart.

#### **2006 Test**

We conducted individual tree tests in lodgepole pine in Lime Kiln Basin near Butte, Montana (Figure 2a) and in whitebark pine on slopes of Mount Edith near Townsend, Montana (Figure 2b). Treatments in both tree species were: (1) two 7.5-gram verbenone pouches, (2) one 7.5gram verbenone pouch and one 10-gram glv pouch, and (3) control, no pouches. There were 50 lodgepole and 41 whitebark pine trees in each treatment. MPB tree baits were placed 5-10 feet from each treated tree. Treatment trees were located at least 2 chains apart. Verbenone and glv pouches were produced by ChemTica, Internacional, S.A. distributed by Synergy Semiochemicals Corp. In whitebark pine, treatments were applied May 30-June 1 and evaluated September 18-19. In lodgepole pine, treatments were applied June 27-28 and evaluated September 25-26.



Figure 2. a. MPB-caused lodgepole pine mortality in Lime Kiln Basin, 2006.



Figure 2. b. MPB-caused mortality in whitebark pine near Mount Edith, 2006.

We conducted individual tree tests in ponderosa pine on Monarch Mountain and whitebark pine on King's Hill (Figure 3). Both sites were located on Lewis and Clark National Forest in the Little Belt Mountains southeast of Great Falls, Montana. Treatments in whitebark pine were: (1) two 7.5-gram verbenone pouches from Synergy Semiochemicals Corp., (2) two 7-gram verbenone pouches from Pherotech, Inc., (3) one 7.5-gram verbenone and one 10-gram glv pouch (Synergy) and (4) no pouches (control). Treatments in ponderosa pine were the same as

whitebark with the addition of a treatment consisting of two glv pouches. There were 40 trees in each treatment, each host. All trees were located at least 2 chains apart. Treatments were randomly assigned. Once again, a standard MPB tree bait (Synergy) was placed 5-10 feet away from each treated tree. Treatments were installed in whitebark pine on June 18-20 and in ponderosa pine on June 25-26. Tests in both species were evaluated September 25-26.



Figure 3. a. Whitebark pine mortality at King's Hill, 2007.



Figure 3. b. Ponderosa pine mortality on Monarch Mountain, 2007.

#### **Levels of MPB-Caused Mortality**

In order to assess MPB infestation levels in project areas, we tallied MPB-caused mortality on 10 variable-radius (BAF 10) plots established near each area: Corona Ridge in 2004, Edith Peak and Lime Kiln in 2006, and Kings Hill and Monarch Mountain in 2007. Unattacked trees, current-year attack, previous-year attack, and older dead trees were recorded. These data were analyzed using the computer program FINDIT, a program used to tabulate insect and disease-caused damage in affected stands (Bentz 2000). Data are summarized in Table 1.

#### **Statistical Analysis**

Numbers of trees with no beetle attacks, pitchouts, strip-attack, and mass-attack were summarized by treatment. Pearson Chi-square test was used to test for significant differences in type of MPB attack among treatments in 2004 and 2006. In 2007, SAS procedure GLIMMIX was used to test for differences between the control and other treatments (SAS 2006).

#### **RESULTS**

#### **Beetle Populations Estimates**

MPB-caused tree mortality in verbenone project areas is shown in Table 1. Mortality ranged from 32 to 67% in areas surveyed. Bark beetle populations are traditionally considered to be at "epidemic" level when beetle-caused mortality exceeds one tree per acre (TPA) per year (Weatherby and Thier 1993). All project areas were at epidemic levels.

Forest- Test	Green Trees >5 inches	Current- Year	% Green Stand	Previous- Year	Older Beetle	Total Percent
Area	<u>2</u> 5 menes d.b.h.	Attacks	Currently	Attacks	Attacks	Killed <sup>1</sup>
	(TPA)	(TPA)	<b>Infested</b>	<u>(TPA)</u>	<u>(TPA</u> )	
Lolo-Corona	82	17	17	56	28	55
Ridge (2004)						
Helena-	107	32	23	82	103	67
<b>Mount Edith</b>						
<u>(2006)</u>						
Beaverhead	140	76	35	19	32	48
Lime Kiln						
<u>(2006</u> )						
L&C-Kings	120	41	25	6	25	38
Hill (2007)						
L&C-Mon.	103	2	2	18	29	32
Mtn. (2007)						

Table 1. Stand data including MPB-caused tree mortality in TPA and percent trees killed (1"Total Percent Killed" includes "Current-Year Attacks," "Previous-Year Attacks," and "Older Beetle Attacks" at time data collected.)

In whitebark pine, trees treated with two 7.5 g verbenone pouches were protected from mass attack nearly 90% of the time (Figure 4). While 77% of control trees were killed, 87% of treated trees survived. Trees that survived include 8% that were partially attacked but not killed. The verbenone treatment was significantly different (p<.001) than the control.

In lodgepole pine, 90% of control trees were mass attacked while about 70% of trees in both

treatments were not (Figure 5). However, 22% of the 5-gram-pouch-treated trees and 25% of the 7.5-gram-pouch-treated trees had strip-attacks. Both treatments were significantly different than controls (p<.001), but not from each other.

Average diameter-at-breast height (d.b.h.) was 14.8 inches in whitebark pine and 10.2 inches in lodgepole pine. D.b.h. was not significantly different between treatments in either host.

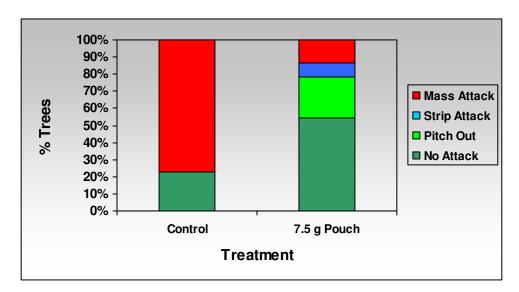


Figure 4. Whitebark pine test, Fisher Peak, 2004, using two 7.5-gram verbenone pouches and no pouches (control) per tree. Verbenone treatment was significantly different (p<.001) than controls.

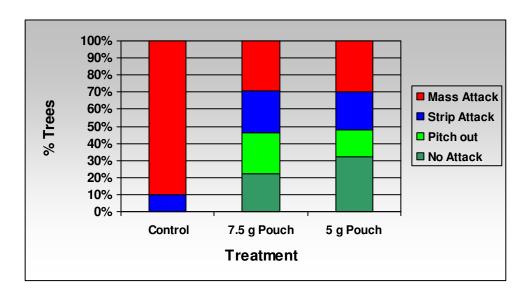


Figure 5. Lodgepole pine test, Corona Ridge, 2004, using two 7.5-gram verbenone pouches, two 5-gram pouches with mid-season replacement, and no pouches (control). Both treatments were significantly different (p<.001) than controls but not from each other.

In whitebark pine, treated trees were protected from mass attack 83-85% of the time while 73% of untreated control trees were killed (Figure 6). Both treatments were significantly different than controls (p<.001) but not from each other. About 6% of treated trees had unsuccessful attacks (pitch outs) and about 16% had stripattacks compared to 2% pitch outs and 5% strip attacks on the control trees.

In lodgepole pine, 76% of control trees were mass attacked compared to 24% of verbenone

treated trees and 10% of verbenone plus glv-treated trees (Figure 7). Both treatments were significantly different than controls (p<.001) but not from each other. About 20 % of verbenone treated trees had pitchouts and 12% had strip-attacks compared to 12% pitchouts and 14% strip attacks on verbenone plus glv-treated trees.

Average d.b.h. of whitebark pine trees was 12.6 inches and lodgepole pines, 10.2 inches. There were no significant differences in d.b.h. between treatments of either host.

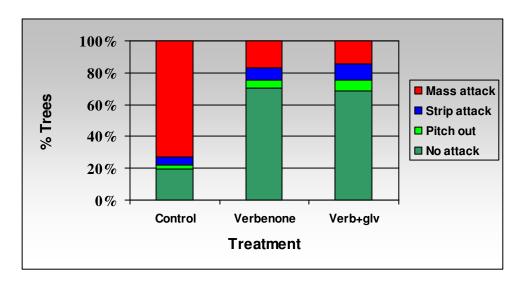


Figure 6. Whitebark pine test, Mount Edith, 2006. Verbenone and verbenone plus glv treatments were significantly different than controls (p < .001) but not from each other.

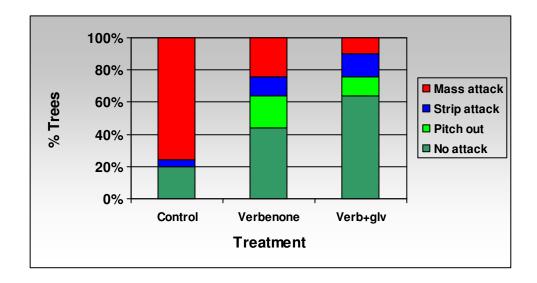


Figure 7. Lodgepole pine test, Lime Kiln, 2006. Treated trees were significantly different than controls (p<.001) but not from each other.

In whitebark pine, nearly 90% of control trees were killed while over 80% of verbenone treated trees had no successful beetle attacks (Figure 8). From 5-10% of verbenone treated trees were strip-attacked. About 75% of trees treated with verbenone and glv had no successful beetle attacks while 17.5% were strip-attacked. All verbenone and verbenone plus glv treatments were significantly different from control trees (p<.001) but not from each other.

In ponderosa pine, 55% of control trees were killed while about 90% of verbenone and

verbenone plus glv-treated trees had no attacks or pitchouts (Figure 9). Almost 40% of trees treated with two glv pouches were mass-attacked. Verbenone and verbenone plus glv treatments were significantly different (p<.001) from controls; however, the 2 glv-pouch treatment was not.

Average d.b.h. of whitebark pine treatment trees was 13.3 inches. Ponderosa pines averaged 14.6 inches d.b.h. D.b.h. was not significantly different between treatments in either host.

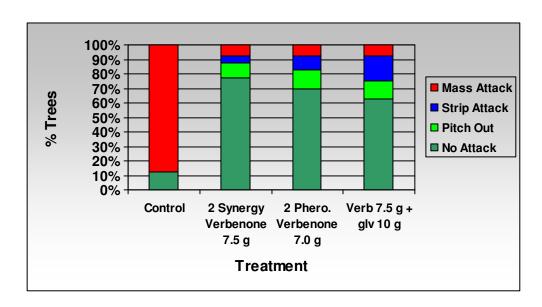


Figure 8. Whitebark pine test, King's Hill, 2007. All treatments were significantly different than controls (p<.001), but not from each other.

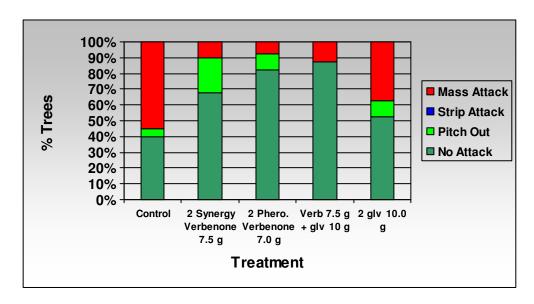


Figure 9. Ponderosa pine test, Monarch Mountain, 2007. Verbenone and verbenone + glv treatments were significantly different from controls, but the 2 glv treatment was not (p<.001).

#### **DISCUSSION**

We have tested verbenone, alone and in combination with glv, against three hosts, and in a variety of environmental settings. While we have not achieved complete protection from MPB attacks with any single treatment, some treatments have been satisfactory enough to warrant further evaluations, and in some cases, operational recommendations.

Our tests found that the following treatments provided the best protection—generally 80% or greater—against MPB attack, when compared to untreated controls:

- Two 7.5-gram Synergy pouches (currently registered [Synergy Semiochemicals, Corp.])
- Two 7-gram Pherotech pouches (currently registered [Contech, Inc.])
- Two 5-gram Pherotech pouches changed at mid-season

Two currently registered 7- or 7.5-gram pouches applied before MPB flight, will provide very acceptable individual-tree protection from MPB attack for one season under most circumstances. Our tests also showed that one 7.5-gram Synergy verbenone pouch plus one 10-gram glv pouch provided reasonably good protection, and likely bears further evaluation.

While this report specifically details "individual-tree" protection, in operational "area" treatments, it has been our experience that applications of verbenone have been more effective when MPB populations are not extreme. Precisely what MPB infestation level would render verbenone treatments ineffective has not been determined. However, verbenone distributors suggest if MPB currently infest more than 15% of the trees in the stand being considered for treatment, most or all infested trees should be removed prior to verbenone application. If that is not possible,

verbenone treatments may not provide sufficient protection to warrant their implementation (John Borden, Contech, Inc.: David Wakarchuk, Synergy Semiochemicals Corp.; personal communication, 2008). How that caveat might apply to individual-tree protection we are not sure; but we note in all but one of our treatment areas, current MPB infestation levels exceeded 15% (Table 1). Unquestionably, treatments are more focused when verbenone is applied at a rate of 2 pouches per tree, compared to 20-40 pouches per acre in an area treatment; but it is prudent to inform verbenone users that high levels of MPB activity may result in less-thanhoped-for results.

We may never achieve the level of individualtree protection with verbenone and/or glv applications that we do with insecticidal preventive treatments, which when properly applied routinely provide 100% protection (Fettig et al. 2006). However, there will often be circumstances under which insecticides cannot be used, or will not be the treatment of choice. In those situations, verbenone alone or in some combination with glv may provide a very satisfactory alternative. To reiterate, in those conditions where MPB populations are very high, e.g, exceeding 15% of the stand currently infested; and most infested trees cannot be verbenone removed in conjunction with application, one may realize poorer results. In any case, it will be incumbent upon us, as Forest Health Protection specialists, to be certain those whom we assist understand that verbenone is not There will be situations a perfect treatment. where it should not be used at all; others only with some ameliorating conditions. Still, it is our conclusion, that almost always, in an effort to protect high-value trees from MPB attack more SO for individual-tree than area protection—using verbenone will be considerably better than doing nothing.

#### **ACKNOWLEDGEMENTS**

Michael Marsden provided statistical analysis. Doug Wulff, Tom Eckberg, Gary Kempton, Brytten Steed, Natalie Angel, John Schwandt, and Bill Cramer provided valuable field assistance.

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